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(21) *Patentansökningsnummer* *0300958-6*
Patent application number

(86) *Ingivningsdatum* *2003-04-02*
Date of filing

Stockholm, 2004-04-14

För Patent- och registreringsverket
For the Patent- and Registration Office

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ANALYTICAL METHOD AND KIT THEREOF

Field of the invention

The present invention relates to a peptide comprising a symmetrical dimethylated arginine, and constitute an immunologic determinant of antibodies present in sera from patients with systemic lupus erythematosus (SLE), and wherein the methylation is a prerequisite for reacting with said antibodies. The invention also relates to the use of said peptide for diagnosis of SLE.

Background of the invention

Systemic rheumatic diseases are characterized by the occurrence of circulating autoantibodies to defined intracellular targets (reviewed in von Mühlen and Tan, 1995). Among the earliest of those autoantibodies to be identified were the anti-Sm, which are closely associated with systemic lupus erythrematosus (SLE) (Tan and Kunkel 1966). Thus, anti-Sm antibodies have been included as one of the American College of Rheumatology classification criteria for this disease (Tan et al., 1982). Apart from autoantibodies targeting the Sm-complex anti-DNA, anti-PCNA, anti-U1-RNP, anti-nucleosome, anti-histone, anti-Ro/SS-A, anti-La/SS-B, anti-ribosomal RNP and anti-phopholipid antibodies are frequently found in patients suffering from SLE (von Mühlen and Tan 1995).

In average anti-Sm reactivity is found in 5-30% of patients with SLE, although the specific frequency will vary depending on the detection system and the ethnicity of the SLE population (Abuaf et al., 1990; Jaekel et al., 2001). The Sm-antigen is part of the spliceosomal complex that catalyzes the splicing of nuclear pre-mRNA (Seraphin, 1995; Lerner et al., 1980). The complex itself comprises at least nine different polypeptides with molecular weights ranging from 9 – 29.5 kDa [B (B1, 28 kDa), B´(B2, 29 kDa), N (B3, 29.5 kDa), D1 (16 kDa), D2 (16.5 kDa), D3 (18 kDa), E (12 kDa), F (11 kDa) and G (9 kDa)] (Hoch, 1994). All of those core proteins can serve as targets of the anti-Sm immune response, most frequently the B and D polypeptides, which are therefore considered the major antigens (Hoch, 1994; Brahms et al., 1997; Ou et al., 1997). However, SmBB´ and

U1 specific RNPs share crossreactive epitopes, consequently SmD is regarded as the most specific Sm-antigen (van Venrooij et al., 1991; Hoch et al., 1999). Within the SmD family the SmD1/D3 pattern is at least four times more common than SmD1/D2/D3 recognition with a pronounced immunoreactivity to SmD1 (Hoch et al., 1999). In epitope-mapping studies, several linear and conformational epitopes have been mapped on the SmB- and D-proteins (Rokeach et al., 1992; Hirakata et al., 1993). On SmD1 and BB´ the major reactivity was predominantly found in the C-terminal extensions (Rokeach et al., 1992; Hirakata et al., 1993; Rokeach and Hoch, 1992). The epitope PPPGMRPP that occurs three times within the C-terminal extensions of SmBB´ was shown to crossreact with other prolin rich structures of spliceosomal autoantigens and of retroviral proteins such as p24 gag of HIV-1 (De Keyser et al., 1992). Follow-up studies and immunization experiments revealed that this motif is consistently the earliest detectable SmBB´ epitope acting as starting point of epitope-spreading events within the BB´ molecule and to the SmD-polypeptides (Arbuckle, 1999; Greidinger and Hoffman, 2001). A recent study identified five linear epitopes on SmD2 and four on SmD3 distributed on the entire molecules (McClain et al., 2002). All of these epitopes share basic properties and are exposed on the surface of the protein rendering them antigenic (McClain et al., 2002). One of the described B-cell epitopes on SmD3 (epitope 4; aa 104-126) displayed close homology to an antigenic region from the SmD1 protein finally leading to crossreactivity (McClain et al., 2002). For diagnostic purposes a synthetic peptide corresponding to the C-terminal extension of SmD1 was used to develop an ELISA system with diagnostic sensitivities and specificities ranging from 36-70% and from 91.7% and 97.2%, respectively (Riemekasten et al., 1998; Jaekel et al., 2001). Recently, it has been shown, that the polypeptides D1, D3 and BB´ contain symmetrical dimethylarginine (sDMA) constituting a major autoepitope within the C-terminus of SmD1 (Brahms et al., 2000; Brahms et al., 2001). In one of these studies a synthetic peptide of SmD1 (aa 95-119) containing sDMA demonstrated significant increased immunoreactivity compared to the non-modified peptide reflecting a conflict to previous data (Riemekasten et al., 1998; Brahms et al., 2000).

In WO 99/11667 a method is described for producing peptides containing methylated arginines and that constitute immunogenic determinants of antibodies present in sera from patients with SLE or Epstein-Barr virus (EBV) and wherein the methylation is a prerequisite for reacting with said antibodies. However, these peptides are generally described and no connection between peptide sequence and ability to diagnose autoimmune disease has been disclosed.

We have now found that our claimed peptide comprising a symmetrical dimethylated arginine is essential for the diagnosis of SLE, and it has surprisingly been shown that this peptide can be used in a highly specific and reliable diagnostic immunoassay for selection of SLE patients.

Summary of the invention

It is an object of the present invention to provide an analytical method for detection of anti-Sm antibodies.

The present inventor has surprisingly found that symmetrical dimethylation of the arginine residue within the SmD3 sequence is crucial for its antigenicity.

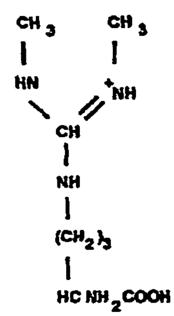
Therefore, in one aspect, the present invention provides a peptide (S33) containing 15-16 amino acids, comprising symmetrical dimethylated arginine (sDMA), that is able to react with antibodies and with said dimethylation being crucial for the reaction between said peptide and said antibodies and wherein said antibodies are present in sera from patients with systemic lupus erythematosus (SLE).

In a second aspect the S33 peptide comprises the amino acid sequence

AARG sDMA GRGMGRGNIF

In a third aspect the symmetric dimethylated arginine has the position 112 in the polypeptide sequence of SmD3.

In a fourth aspect the S33 peptide comprises a symmetric dimethylated arginine with the structure



In a fifth aspect the invention is a method for use of the S33 peptide for in vitro diagnosis of SLE.

In a sixth aspect the invention is a kit for use of the S33 peptide for in vitro diagnosis of SLE.

Brief description of the drawings

Figure 1. Epitope analysis of SmD1 and SmD3. C-terminal extensions of SmD1 (a) and SmD3 (b) were synthesized as peptide arrays (15mers; aa offset) and probed with patient sera. Immunoreactive peptide no. 77 was further tested as mimotope variants (c).

Figure 2. Assay performance characteristics of the new anti-S33 assay. Intra- and interassay variability a.), linearity (b.), and Receiver Operating Characteristic ROC-analysis including Positive Predictive Value (PPV), Negative Predictive Value (NPV) and efficiency at different cut-offs (c.).

Detailed description of the invention

Example 1

Serum samples

Sera (n=451) were collected from patients suffering from systemic lupus erythrematosus (SLE; n=101), rheumatoid arthritis (RA, n=36), Sjögren syndrome (SS, n=24); mixed

connective tissue disease (MCTD, n=10), scleroderma (SSc, n=9) and polymyositis / dermatomyositis (PM/DM, n=2). All patients were classified according to the ACR-criteria for each disease (Tan et al., 1982; Arnett et al., 1988). The SLE group contained 34 samples from white patients, 51 from black patients, 5 from Hispanic patients, 1 sample from an east Indian patient and 1 from an oriental patient. Four patients had unknown etiology. Among these patients 13 were male and 86 female (2 unknown), with a mean age of 40 years (range 16-80 years). To further assess the assay specificity, we analyzed a group of sera from patients with infection diseases (n=77) including hepatitis-C (HCV; n=30), cytomegalo (CMV; n=22) and Epstein-Barr Virus (EBV; n=25) as well as from 192 healthy blood donors. In addition, ANA reference sera were tested (Smolen et al., 1997; Tan et al., 1999; Tan et al., 2001). All sera were stored at –80°C until use. For epitope-mapping a panel of five sera containing anti-Sm antibodies was used. As negative controls autoimmune sera with other antibody specificities than anti-Sm were selected.

Serological characterization of the patient sera. All autoimmune patient sera were tested for autoantibodies to histones, dsDNA and the Sm-complex using quantitative Varelisas (Pharmacia Diagnostics, Freiburg, Germany). SLE sera and samples, which demonstrated unexpected results were also measured in the semi quantitative ANA-Split ELISA Kit (Pharmacia, Freiburg, Germany). The latter assay contains the autoantigens U1-68kDa, U1-A, U1-C, SmBB´, SmD, Ro-52, Ro-60 and La. All ELISAs were performed according to the instructions of use.

<u>Example 2</u>

Epitope-mapping with immobilized oligopeptides

The published sequences of SmD1, P13641, (Rokeach et al., 1988) and SmD3, P43331, (Lehmeier et al., 1994) were used to synthesize overlapping 15mer peptides with a pipetting robot according to the protocol described by Gausepohl and Behn (2002). The C-terminal extensions of both polypeptides were synthesized with an offset of 2 amino acids (13 amino acids overlap). Each arginine containing peptide was synthesized as three variants, with natural arginine, with sDMA or with asymmetrical dimethylarginine (asDMA) at the respective positions. Later on, a highly reactive peptide of SmD3 was

synthesized with certain combinations of natural arginine and sDMA. Following completion of the peptide synthesis non-specific binding sites were blocked by over-night incubation of the membranes in blocking buffer (BB) at room temperature (RT). After one washing step membranes were incubated with serum samples at a dilution of 1:100 in BB for 2 h at RT. Unbound antibodies were removed by three washing steps . For detection peroxidase conjugated goat-anti-human IgG antibody was diluted 1:5000 in BB and incubated for 75 min (RT). Superfluous secondary antibodies were removed by three washing steps . Finally, bound antibodies were visualized using the enhanced chemoluminiscence (ECL) detection-system. Assay conditions were used under which negative sera showed no reactivity.

Example 3

S33-peptide ELISA

Preparation of ELISA-plates. The lyophilized S33 peptide was used to prepare a stock solution of 10µg/µl , which was stored in aliquots at –20°C until use. Binding of the peptide to ELISA plates was carried out using 2.5µg/ml of the peptide in coating buffer in a final volume of 120µl per well. The coating procedure was carried out at 15°C for 20h. Unspecific binding sites were blocked with blocking solution . After discarding the blocking solution solid phases were dried at 37°C for 2h and sealed.

The assay was performed according to the general protocol of the Varelisa system (Pharmacia Diagnostics, Freiburg). Blood donors demonstrated a reactivity range of 0.4-11.5 U/ml resulting in a mean value of 2.2 U/ml and a SD of 1.2 U/ml. The cut-off was technically set to 13 U/ml after ROC-analysis. PPVs and NPVs were calculated at different cut-off values.

Precision and reproducibility. Measurements of imprecision (inter- and intra-assay variability) were performed with 4 and 6 replicates, respectively. To assess precision of the anti-S33 peptide ELISA suitable anti-Sm sera, a low value sample (L); a medium value sample (M) and a high value sample (H) were assayed in five independent runs on one day (inter-assay), or in a single run (intra-assay). For within-run precision L, M and H were

measured in six replicates on one solid phase. The precision data was calculated using ANOVA analysis.

Linearity. The linearity was analyzed by testing dilutions (1:1; 2:3; 1:2; 1:4; 1:8; 1:16; 1:32) of the highest standard point (S6) and of the high value sample from the precision analysis (H). For each dilution point, a ratio of the measured reactivity to the expected value was calculated, and 1 was subtracted from this quotient.

Results

Epitope fine-mapping of the C-terminal extensions of SmD1 and D3. To evaluate the effect of arginine-dimethylation on the antigenicity of SmD1 and SmD3 and to map relevant epitopes on both polypeptides a panel of anti-Sm sera was tested with peptide arrays (15mer, 2 offset) covering the C-terminal region of SmD1 (P13641) and SmD3 (P43331).

The results show that dimethylation of arginine residues affects the binding of anti-Sm antibodies to C-terminal SmD1- and D3 polypeptides, significantly (see figure 1). All anti-SmD sera demonstrated an increased binding to SmD1 peptides containing the symmetrical form of dimethylarginine (sDMA). Especially the peptides that consist of glycine and DMA repeats, exclusively showed a strong reactivity with the antibodies (peptide no. 9, 10). Nevertheless, SmD1 polypeptides containing DMA represent a rather unspecific substrate for anti-Sm antibodies since they were also target of anti-centromere antibodies (ACA). Interestingly, those ACA bound also to peptides containing the asymmetrical form of DMA.

Binding experiments with peptides derived from SmD3 showed similar results. Only SmD3 peptides containing sDMA reacted with anti-Sm antibodies confirming the importance of the symmetric methylation of arginine residues (see figure 1b). In contrast to SmD1, no control serum demonstrated antibody binding to SmD3 derived peptides reflecting a high specificity. One particular peptide (no. 77, ^{108}AA*sdR*G*sdR*G*sdR*GMG*sdR*GNIF^{122}) was strongly recognized by three out of five anti-Sm sera. Using a mutational analysis in which arginine residues of ^{108}AARGRGRGMGRGNIF^{122} were successively replaced by sDMA we were able to

show that a mimotope peptide with a single dimethylated arginine residue at position 112 displayed immunoreactivity with all of the five anti-Sm sera but not with the controls (see figure 1c.). Thus, by introducing only one sDMA and at a defined position (amino acid 112) of SmD3, it was possible to increase the sensitivity of this peptide (^{108}AARG*sdR*GRGMGRGNIF^{122}; S33) without a loss in specificity. This candidate peptide was subsequently synthesized as soluble antigen and used as substrate in ELISA.

Immunoserologic characterization of the SLE patient group. Sera from SLE patients were tested for U1-68kD, U1-A, U1-C, SmBB´, SmD, Ro-52/SS-A, Ro-60/SS-A, La/SS-B, histone dsDNA and ß2-glycoprotein reactivity (Split ANA-Profil research assay, Pharmacia Diagnostics, Freiburg, Germany). The prevalence of the different autoantibodies was found in a good agreement to previous studies (Jaekel et al., 2001). Thus, with regard to their autoantibody profiles, the SLE cohort seems to be a representative SLE population. Results of the measurements of the SLE panel are summarized in table 1.

Table 1. Prevalence (%) of clinically relevant autoantibody specificities in patients suffering from SLE (n=101)

Autoantibodies to										
U1-68	**U1-A**	**U1-C**	**SmBB´**	**SmD**	**Ro-52**	**Ro-60**	**La**	**Histone**	**dsDNA**	**β2-Glycoprotein**
15.8	24.8	25.7	21.8	15.8	21.8	47.5	21.8	37.6	51	17

Anti-S33 peptide ELISA

A 15 amino acid soluble peptide displaying highest sensitivity and specificity in the SPOT-assay (^{108}AARG*sdR*GRGMGRGNIF^{122}) was synthesized for technical reasons with an additional Cys at the C-terminus. This peptide was subsequently used to develop an ELISA system based on the general protocol of the Varelisa tests (Pharmacia, Freiburg, Germany).

Assay performance characteristic. To evaluate the assay performance characteristics precision, reproducibility and linearity were analyzed. The intra- and interassay variability (CV%) of three samples were found ranging from 1.82 to 6.52% and from 2.27 to 7.42%, respectively. Dilution series of two samples demonstrated a linear range on five subsequent dilutions (>20% deviation). For the cut-off definition a ROC- analysis was performed with SLE and control sera. The assay performance characteristics of the new anti-S33-test including intra- and interassay variability (a.), linearity (b.), ROC-analysis, PPV, NPV and efficiency (c.) are summarized in figure 2 (a. – c.).

For the evaluation of the diagnostic relevance of the new test a technical cut-off of 13U/ml was used to combine high specificity with moderate sensitivity. Sera from 101 SLE patients, from 81 autoimmune patients diagnosed differently than SLE, from 77 patients with infection diseases and from 192 human normal donors were analyzed in the new ELISA system. 15 SLE patients (14.9%) were tested positive for anti-S33 antibodies displaying a significantly increased reactivity of up to 952 U/ml with a mean value of 41.11 U/ml (SD = 156.72 U/ml). Patients from related disorders demonstrated a significant reduced reactivity in the new ELISA system (mean 3.36 U/ml). Only one patient of the RA group was assayed positive (24.6 U/ml). None of the remaining controls including patients suffering from SSc (n=10), PM/DM (n=2), MCTD (n=10) or infection diseases (n=77) showed reactivity to the S33 peptide. The serum samples from patients with infectious diseases demonstrated a reduced reactivity (mean 0.67 U/ml; top value 3.3 U/ml), even when compared to the healthy donors (mean 2.21 U/ml; top value 11.5 U/ml). The top value of the infectious disease sera was found in the EBV group. Results are summarized in Table 2.

Table 2. Results of ELISA using S33 with SLE and various control sera

	No. (%) of anti-S33-positve sera	Mean value (U/ml)	Top value (U/ml)
SLE (n=101)	15 (14.9)	41.11	952
Rheumatic diseases (81)	1 (1.2)	3.36	24.6
RA (36)	1 (2.8)	2.77	24.6
pSS (24)	0	1.86	3.9
MCTD (10)	0	4.39	12.8
SSc (9)	0	2.89	4.3
PM/DM (2)	0	4.85	6.4
Infectious diseases (77)	0	0.67	3.3
HCV (30)	0	0.42	1.1
CMV (22)	0	0.8	3.2
EBV (25)	0	0.78	3.3
Healthy individuals (192)	0	2.21	11.5

In summary, 15 samples of the SLE group (n=101) and only one serum of the controls (n=350, 0.3%) was tested positive resulting in a diagnostic specificity of 99.7% and a sensitivity of 14.9%. PPV and NPV, as well as the diagnostic efficiency was calculated at 93.7%, 80.2% and 80.7%, respectively (see figure 2 c.). These data indicate that anti-S33 antibodies appear to be exclusively present in sera from SLE patients.

Apart from the anti-S33 peptide reactivity the false positive RA sample contains high titers of antibodies to the U1-RNPs- 68kDa (ratio 4.5), U1-C (ratio 9.4) and histones (133.8 U/ml) (see table 3). Anti-SmBB´and anti-SmD titers as determined by ELISA were elevated when compared to the controls, but still below the cut-off values (see table 3).

Table 3. Autoantibody-profile of the false positive RA patient in the new S33 peptide assay

Serum ID #	Control group	U1-68kD*	U1-A*	U1-C*	SmBB′*	SmD*	Ro-52*	Ro-60*	La*	histone[1] [U/ml]	dsDN [U/ml
R15	Ra	4.5	0.6	9.2	0.8	0.8	0.2	0.7	0.5	133.8	15.6

*semiquantitative Assay (ANA-Split); cut-off >1.4

[1]cut-off (30U/ml)

[2]cut-off (55U/ml)

Correlation to other autoantibodies. With regard to possible existing correlations between anti-S33 antibodies and other autoantibody species, statistically significant correlations were found (see table 4). The anti-S33 peptide reactivity was correlated to U1-68kDa (p= 0.0335), U1-A (p < 0.0001), U1-C (p < 0.0001) SmBB′ (p < 0.0001), SmD (p < 0.0001), dsDNA (p < 0.0001) and histone (p < 0.0001), but not to Ro-52 (p= 0.2192), Ro-60 (p= 0.2212) and La (p= 0.8785) (see table 4).

Table 4. Association between anti-S33 positivity and other Aab species in SLE

Aab to	U1-68kD	U1-A	U1-C	SmBB′	SmD	Ro-52	Ro-60	La	histone	dsDNA	β2-Glycoprotein
S33 +	8/16	12/25	11/26	11/22	11/16	5/22	12/48	4/22	10/38	13/51	4/16
	50%	48%	42.3%	50%	68.8%	22.7%	25%	18.2%	26.3%	25.5%	25%
2-tailed p	0.0335*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.2192	0.2212	0.8785	<0.0001*	<0.0001*	0.3792

Pearson correlation; * Statistically significant

Looking at the reactivity towards the Sm-complex, five samples reacting with the purified SmD antigen was found, but not with the S33 peptide. The remaining 11 SmD positive sera (68.8%) were also tested positive in the new anti-S33 peptide ELISA. Interestingly, among the anti-S33 positive samples, 4 patients (#89, #92, #20627, #9811) were found, all anti-SmD negative showing anti-S33 peptide reactivities of 15.4, 21.3, 41.3 and 13.9 units, respectively.

Correlation to etiology and clinical data. Looking at a possible correlation of autoantibody specificities to the etiology of the patients statistically significant association of autoantibodies was found to U1-68kDa ($p = 0.002$), U1-A ($p < 0.0001$), U1-C ($p = 0.0002$), SmBB′ ($p = 0.0004$), SmD ($p = 0.002$) and dsDNA ($p = 0.0128$) with black SLE patients. The other autoantibody species including S33 ($p = 0.0253$), Ro-52 ($p = 0.8023$), Ro-60 ($p = 0.0399$), La ($p = 0.7137$) and histones ($p = 0.9831$) were statistically not associated with the etiology of the patients under investigation (data not shown). Among the Sm-markers we found the following order of correlation to black etiology of the patients: SmD ($p = 0.0002$) > SmBB′ ($p = 0.0004$) > SmD ($p = 0.002$) > S33 ($p = 0.0253$). No significant correlation was observed between S33 reactivity and renal ($p = 0.2810$) or CNS involvement ($p = 0.5066$).

In the presented examples the anti-Sm immune response have been analyzed towards the Sm antigens D1 and D3, which are considered to be the SLE specific polypeptides (van Venrooji et al., 1991; Hoch et al., 1999). Using immobilized peptides it has been shown that symmetric dimethylation of arginine residues plays an important role in the formation of the major B-cell epitopes on both autoantigens. This observation was found in a good agreement to the result of Brahms et al. (2000) and thus contradictory to the findings of Riemekasten and colleagues (1998). In addition, it was found that with peptides as previously decribed the specificity of SmD3 peptides was higher than of those derived from SmD1.

McClain and colleagues (2002) described four antigenic regions on SmD3 of which antigenic region 4 covers the area 104-126. In this invention peptides synthesized on pins were subjected to analysis but without using the modified form of arginine. In the present invention reactivity within this region was only found in case natural arginine was replaced by sDMA. These contradictory results might be explained by the use of different sera, methology and / or by the varying peptide length. Three out of five sera specifically recognized the peptide ^{108}AA*sdR*G*sdR*G*sdR*GMG*sdR*GNIF^{122} of this example. Interestingly, the dimethylation of only one arginine and at a defined position (aa 112) could further increase the sensitivity of this particular peptide without a loss in specificity. Based on this data a candidate peptide was used (^{108}AARG*sdR*GRGMGRGNIF^{122}) to develop an ELISA system. The new anti-Sm assay (anti-S33) demonstrated a sensitivity of 14.9% and a specificity of 99.7% for lupus resulting in a high positive (PPV; 93.7%) and negative predictive value (NPV; 80.2%) and thus a high diagnostic efficiency (80.7%). Therefore this test offers new opportunities for the diagnosis of systemic lupus erythrematosus.
Looking at the biochemical properties of the identified Sm-epitopes reveals that the pI can be regarded as predictor of antigenicity on the Sm-complex. On U1-RNP-A, SmB´ and D1, the average pI of antigenic regions was 10.4 (nonantigenic 6.0) and on SmD2 and D3 more than pIs 9.0 (McClain et al., 2002). These inventive findings fit well to the high pI of the S33 peptide (>12.88). Whether the basic character simply increases the probability of surface exposure of these regions and thus the accessibility to antibodies has to be further investigated.

EBV, EBNA and anti-SmD antibodies. Epitope-mapping studies on SmD1 have identified an epitope-motif (aa 95-119) that cross-reacts with a homologue sequence 35-58 of the Epstein-Barr virus nuclear antigen 1 (EBNA-1) (Sabbatini et al., 1993; Sabbatini et al., 1993; Marchini et al., 1994). A more recent study has shown that this epitope also cross-reacts with a homologue region of SmD3 containing glycine arginine repeats (RGRGRGMGR) (McClain et al., 2002). Moreover it became evident that GPRR (aa 114-119 on SmD1) represents a common cross-reactive autoepitope motif, which is present not

only on EBNA-1, but also on a variety of autoantigens including CENP-A, B, C, SmBB′, SmD1 and Ro-52, to term only a few (Mahler et al., 2001). Thus patients suffering from infectious mononucleosis or SLE related disorders might be tested false positive in ELISAs using the C-terminal extensions of SmD1 or SmD3. Furthermore, several studies have suggested an influence of EBV on the development of Lupus-like conditions (James et al., 1997). Therefore, it is considered that the use of EBV positive sera as controls is an important finding towards a highly specific and reliable anti-SmD immunoassay. Among the 25 EBV disease controls presented, no false positive sample was found confirming the suggested high specificity of the anti-S33-abs assay. Unfortunately, Riemekasten and colleagues (1998) did not include this patient group in the evaluation of their test.

Correlations to other autoantibody species. Overlapping reactivity between DNA and Sm antigens has been reported in several publications (Bloom et al., 1993; Reichlin et al., 1994; Zhang et al., 1995). While in these studies full-length SmD was used, in present invention, there was also a correlation of the anti-dsDNA and anti-S33 reactivity ($p < 0.0001$). Apart from DNA the present invention also shows a positive correlation of anti-S33 to U1-68 ($p < 0.0001$), U1-A ($p < 0.0001$), U1-C ($p < 0.0001$), SmBB′ ($p < 0.0001$), Sm ($p < 0.0001$) and SmD ($p < 0.0001$), but not to histones ($p = 0.0259$), La ($p = 0.8747$), Ro-52 ($p = 0.4034$) and Ro-60 ($p = 0.0143$). Whether the observed associations are caused by cross-reactivity or by different autoantibody species that often occur simultaneously, remains unclear. Further studies have to be addressed to shed more light on this issue.

Riemekasten vs Brahms. The obvious conflict between the results of Riemekasten et al. and Brahms et al. might be explained by the existence of different epitopes on the C-terminal extensions of SmD1. The peptide aa 83-119 (Riemekasten et al., 1998) may form a conformational epitope, whereas the shorter peptides used in the second study contain linear, sDMA dependent binding sites (Brahms et al., 2000). Furthermore, the reduced reactivity against the full-length SmD1 (Riemekasten et al., 1998), compared to $SmD1_{83-119}$ peptide, suggests that this peptide epitope represents a cryptic structure. This observation raises the question, which epitopes are "seen" *in vivo* and which ones play the central role

in the pathogenesis of SLE. In a recent study it became evident, that the injection of $SmD1_{83\text{-}119}$ fused to a carrier protein is able to accelerate the pathogenic process of Lupus-prone mice (Riemekasten et al., 2001).

"Rhupus"-Syndrom. Rheumatoid Arthritis (RA) and systemic lupus erythremtosus (SLE) are related disorders with an autoimmune etiology. Both diseases are accompanied by the occurrence of self-reactive antibodies to defined structures. Several studies have reported overlap syndromes between RA and Lupus, which were therefore sometimes called the "Rhupus"-Syndrom (Miyachi and Tan, 1979; Panush et al., 1988; Brand et al., 1992). In the presented examples one patient was found within the RA group who demonstrated anti-S33 reactivity (24.6 U/ml). Whether this result reflects a false positive testing or wether autoantibodies to the S33 peptide represent a precursor of lupus-like conditions remains unclear and has to be investigated.

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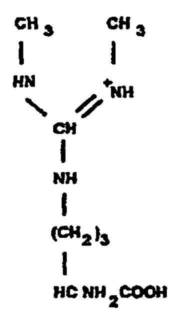
Claims

1) A peptide (S33) containing 15-16 amino acids, comprising symmetrical dimethylated arginine (sDMA), that is able to react with antibodies which are present in sera from patients with systemic lupus erythematosus (SLE).

2) The S33 peptide according to claim 1 comprising the amino acid sequence AARG sdRGRGMGRGNIF

3) A peptide according to claims 1 and 2 where the dimethylated arginine has the position 112 in the polypeptide sequence of SmD3.

4) The peptide according to claims 1 or 2 or 3, wherein the structure of the symmetric dimethylated arginine is



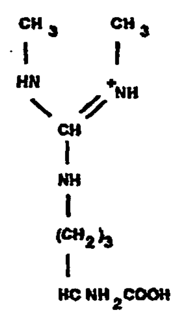
5) Use of a peptide (S33) containing 15-16 amino acids, comprising symmetrical dimethylated arginine (sDMA), that is able to react with antibodies that are present in sera from patients with systemic lupus erythematosus (SLE) for diagnosis of SLE patients.

6) A method for use according to claim 5, wherein said peptide is used for in vitro diagnosis of SLE.

7) A method for use according to claims 5 or 6, wherein said peptide comprises the amino acid sequence
AARG sdRGRGMGRGNIF

8) Use of a peptide according to claims 5 or 6 or 7 where the dimethylated arginine has the position 112 in the polypeptide sequence of SmD3.

9) A method for use according to any of claims 5 or 6 or 7 or 8, wherein the structure of the symmetric dimethylated arginine is



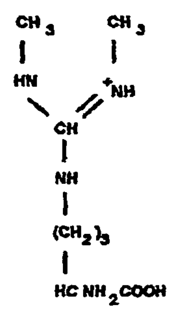
10) A kit for detection of antibodies, comprising a peptide (S33) of 15-16 amino acids of which one is a symmetrical dimethylated arginine (sDMA), and is able to react with said antibodies that are present in sera from patients with systemic lupus erythematosus (SLE).

11) A kit for use according to claim 10, wherein said peptide is used for in vitro diagnosis of SLE.

12) A kit for use according to claims 10 or 11, wherein said peptide comprises the amino acid sequence
AARG sdRGRGMGRGNIF

13) A kit for use of a peptide according to claims 10 or 11 or 12 where the dimethylated arginine has the position 112 in the polypeptide sequence of SmD3.

14) A kit for use according to any of claims 10 or 11 or 12 or 13, wherein the structure of the symmetric dimethylated arginine is



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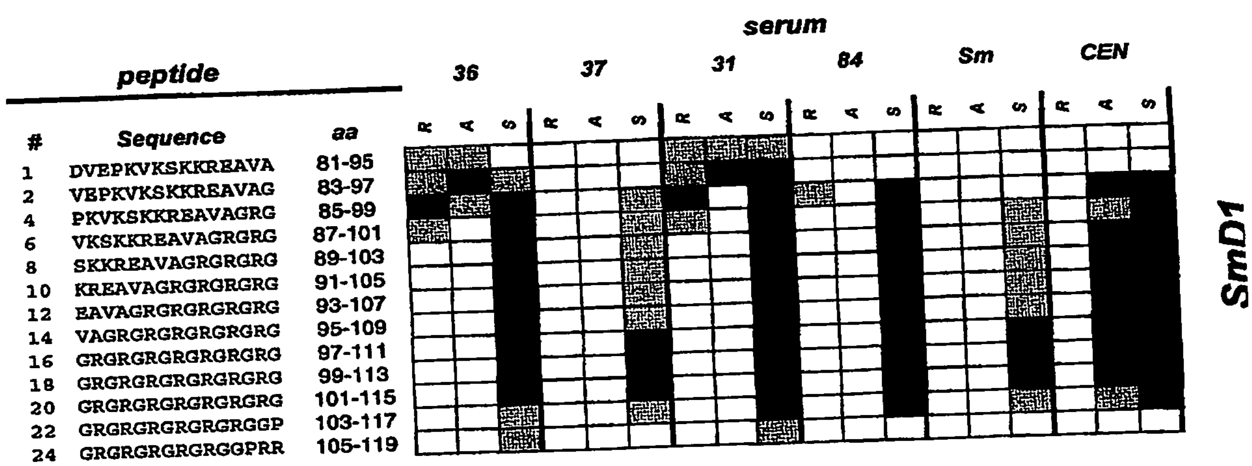
Abstract

The present invention relates to a peptide comprising a symmetrical dimethylated arginine, and constitute an immunologic determinant of antibodies present in sera from patients with systemic lupus erythematosus (SLE), and wherein the methylation is a prerequisite for reacting with said antibodies. The invention also relates to the use of said peptide for diagnosis of SLE.

Drawings

1. a.)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37
31
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Sm
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16 GRGRGRGRGRGRGRG 97-111
18 GRGRGRGRGRGRGRG 99-113
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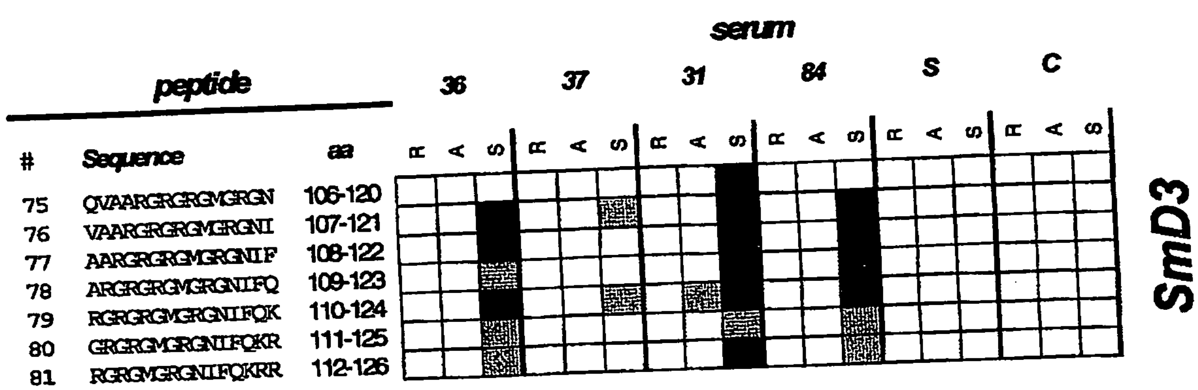
SmD1

1. b.)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peptide
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31
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S
C
#
Sequence
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79 RGRGRGMGRGNIFQK 110-124
80 GRGRGMGRGNIFQKR 111-125
81 RGRGMGRGNIFQKRR 112-126
SmD3

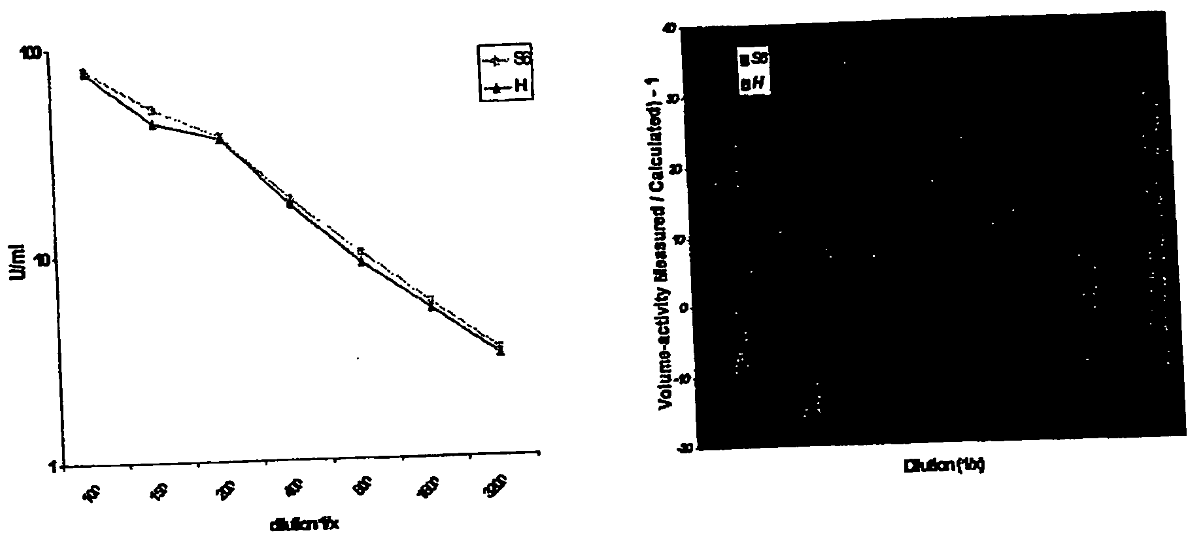
1. c.)


36
37
31
84
Smith
CEN-0006
AARGRGRGMGRG
AAsGRGRGMGRG
AARGsGRGMGRG
AARGRGsGMGRG
AARGRGRGMGsG

2. a.

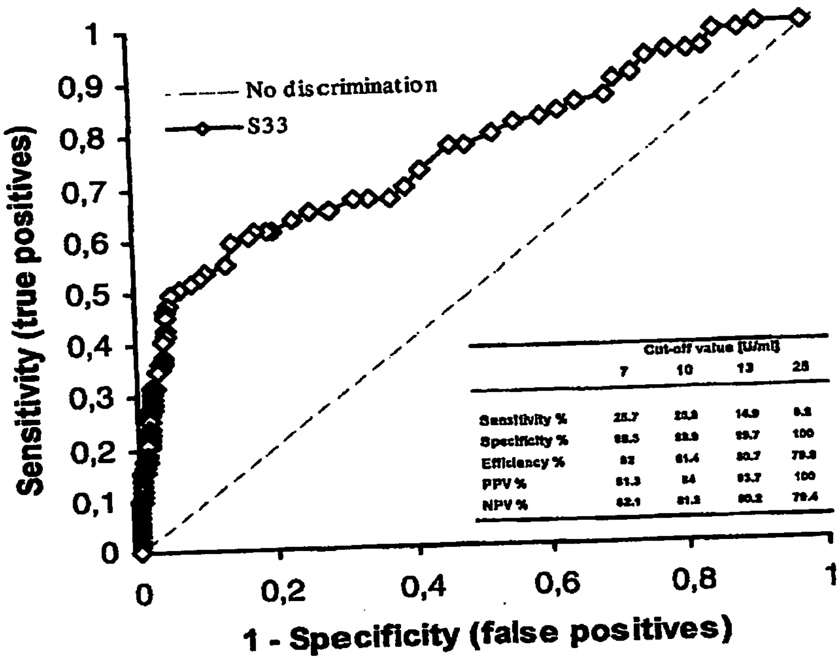
	Antibody concentration		
	Low	Medium	High
Intra-assay			
Mean [U/ml]	7.1	36.3	83.8
CV (%)	1.82	3.79	6.52
Inter-assay			
Mean [U/ml]	7.48	36.54	91
CV (%)	3.97	7.42	2.27

2. b.



100
10
1
U/ml
dilution 1/x
S6
H
Volume-activity Measured / Calculated) - 1
Dilution (1/x)

2. c.



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0,5
0,4
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0,1
0
Sensitivity (true positives)
No discrimination
S33
Cut-off value [U/ml]
7
10
13
28
Sensitivity %
Specificity %
Efficiency %
PPV %
NPV %
0
0,2
0,4
0,6
0,8
1
1 - Specificity (false positives)

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